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CIRCULATORY RESPONSES OF THE FETAL LAMB *IN UTERO* TO INCREASE OF INTRAUTERINE PRESSURE

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Received for publication June 22, 1955

The fact that the fetal heart rate slows when the uterus contracts strongly is common knowledge to obstetricians. Persistence of the bradycardia throughout the period of uterine diastole is generally regarded as an unfavorable clinical sign, for it is believed that the fetus then has been subjected to excessive stress. We lack information, however, concerning the physiological nature of such a fetal circulatory response to increased intrauterine pressure. Recent development of a technic for catheterization of the fetal circulation of the monkey and lamb *in utero* while the membranes are still intact, by Reynolds, Paul and Huggett (1), has opened for study the problem of the circulatory responses of the fetus to changes in intrauterine pressure.

Experiments made with the non-breathing fetus delivered by Caesarean section with the placental circulation intact suggest the kind of physiological mechanisms that may be involved when intrauterine pressure increases. For example, when the umbilical arteries alone are temporarily occluded, a rise in fetal blood pressure and immediate bradycardia occur. The latter is inhibited by double vagotomy, Reynolds, (2). Even after vagotomy, a delayed bradycardia after thirty to forty-five seconds develops upon occlusion of the umbilical arteries. The immediate bradycardia, therefore, is a reflex inhibition, the delayed bradycardia a direct effect of hypoxia upon cardiac muscle.

A second type of bradycardia has been demonstrated in the fetal lamb. When the two umbilical veins are temporarily occluded, there is a prompt reflex bradycardia which is abolished by vagotomy, Reynolds (2). Unlike the bradycardia following arterial occlusion, this second type is associated with a fall of blood pressure. It is not, therefore, an aortic depressor reflex. It may be due to a central hypoxia which serves to activate the vagal centers directly or it may result from activation of other, heretofore unsuspected peripheral receptors. The carotid body, it has been shown is not involved, Reynolds (3).

In the present study, the blood pressure and heart rate responses were observed as intrauterine pressure was changed by placing weights upon the uterus and subsequently removing them or by injecting Pitocin. The observations show that the circulatory changes which occur in the fetus in response to such stimuli are mediated through the activity of the vagus nerve. The changes

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in blood pressure which occur during rhythmic uterine contractions are likewise noted. Finally, the relation of the bradycardia to central hypoxia is demonstrated.

One might expect *a priori* that a given increment of intrauterine pressure would give rise to an exactly equal increment of blood pressure. If such be the case, the fetus within the uterus behaves as part of a physical fluid system. There are other possibilities, however, owing to the fact that the fetus is a physiological organism with a set of developing homeostatic regulating mechanisms which permit it to respond to various kinds of stressful stimuli. The conclusion does not necessarily follow, therefore, that the blood pressure changes will equal the pressure changes which occur.

PROCEDURES

Animals. Eight grade ewes were employed. The fetuses were 100 to 120 days of gestation age. The ewes were lightly anesthetized with Dial-urethane given intravenously, augmented by ether as necessary for operative procedures. The uterus was exposed by a mid-line incision and the area of maximum distribution of placental cotyledons near the mesometrium determined. An incision was made into the uterus at this point with bleeding prevented by either ligation or the use of broad-nosed (Allis) clamps. The chorion adjacent to the incision was gently stripped and a large branch of an umbilical artery identified by the blue color and the quality of pulsation. It was then catheterized for blood pressure recording by a Statham transducer in the manner described in a recent publication, Reynolds, Paul and Huggett (1).

A catheter connected to a strain-gage pressure transducer was passed into the amniotic sac, through a purse-string suture. This was tied in place without loss of fluid for the purpose of recording intrauterine pressure. Maternal carotid blood pressure was recorded through a third Statham pressure transducer.

All three recording circuits were connected into a three-channel Honeywell-Brown intermittent recorder. While this is a suitably sensitive instrument, its speed of recording is such that all pressures had to be damped down so that mean pressures only were recorded. The paper moved at a speed of one centimeter per minute. Fetal and maternal heart rates were counted for fifteen-second intervals by observing the oscillations of a small air bubble trapped in the plastic tubing connected with each strain gage. The figure for each interval was called out and recorded immediately beside the blood pressure record by a second party seated at the recorder. The paper of the recorder moved in such a way that the record had to be read from right to left. Therefore, those tracings that are reproduced here have been copied on tracing paper and reversed photographically to permit reading from left to right. The strain gages were calibrated against a mercury manometer at the end of each experiment.

Procedures. Two methods were used to increase intrauterine pressure. One was passive and involved the placing of weights upon the uterus or manual compression of the uterus. The weights employed were sand bags weighing 1000 grams, 1500 grams, 3000 grams, and 5000 grams. The other was active, by the initiation of a strong uterine contraction by the intravenous injection of Pitocin into the ewe. Pitocin was given in single injections of 0.5 to 2.0 ml., each by jugular vein. The smaller doses were administered slowly, and the larger doses were given rapidly, in an effort to evoke contractions of different intensities.

When all operative procedures were complete, a period of continuous recording lasting twenty to thirty minutes was obtained. Then, according to the design of the experiment for

the day, weights were balanced on the uterus for three or nine minutes, or Pitocin was injected. After each maneuver, ample time was allowed to elapse in order to permit recovery and a steady state of the fetal circulation to be established. This was about twenty to thirty minutes. After the use of Pitocin, rhythmic uterine contractions commonly persisted, but they did not modify the subsequent experimental observations.

In order to assess the extent to which cardiovascular responses in the fetus are the result of vagal activity, atropine was injected directly into the fetal circulation to effect vagal release. In a further attempt to analyze the nature of the fetal cardiovascular response, the effects of having the ewe breathe 100 per cent nitrogen or 100 per cent oxygen were determined. In this way, it could be seen whether simple hypoxia simulated the effects of increased intrauterine pressure. Similarly, it could be established whether elevation of oxygen tension in the tissues reduced or prevented the cardiovascular responses of the fetus to increase of intrarauterine pressure.

Two to fifteen individual observations were made on any one fetus, with an average of five or six tests per fetus. In addition, responses of seven ewes to asphyxia were determined for the purpose of comparison with the fetal response to hypoxia.

RESULTS

Rhythmic uterine contractions. Several sequences of rhythmic contractions were studied in order to compare the increase in fetal blood pressure with the increase of uterine pressure at the same time. One series of eighteen rather weak contractions was typical. Twice, the pressures were equal, seven times the rise in fetal blood pressure exceeded slightly the rise in amniotic fluid pressure, and nine times it was less. For the series, however, a mean increase of 4.6 (σ1.03) mm mercury occurred in amniotic fluid pressure, and 4.5 (σ1.46) occurred in fetal blood pressure. Actually, this is probably only a random difference since the pressure scales employed did not permit reading more closely than to one millimeter of mercury. It appears, therefore, that with small rhythmic uterine contractions, the rise in fetal blood pressure is equal to that in the surrounding amniotic fluid.

Manual compression of the uterus. This was accomplished by four hands clasping and pressing simultaneously upon the uterus for a period of three to five minutes. Fetal heart rates were not counted during this procedure.

It was found that in one fetus of 105 days gestation age the pressures of two different magnitudes produced two different levels of increase of blood pressure. Thus an increase of three millimeters of pressure in the amniotic fluid was associated with a rise of ten millimeters of mercury in fetal blood pressure, while a rise of eight millimeters of mercury in amniotic fluid pressure was associated with an increase of twenty-one millimeters of mercury in fetal blood pressure. In the first instance, the blood pressure: amniotic fluid pressure ratio was 3.3; in the latter, 2.5. Clearly, the rise in blood pressure exceeded the rise in environmental (amniotic) pressure in the two cases, but the relative amounts of increase were different. The conclusion follows that the response of the fetal circulation to a marked change of pressure in the amniotic fluid is not constantly

related to the magnitude of the stimulus. Heart rates were therefore counted in order to see whether this aspect of cardiovascular activity contributed to the response.

Application of weights to the uterus. In fetuses of the age used, a characteristic response of the fetal circulation has been observed upon application of weights to the uterus. There is, of course, an increase of pressure in the amniotic fluid and in fetal blood pressure as well. The relation of one to the other will be discussed below. The character of the changes in fetal heart rate requires comment first.

The fetal heart rate decreases upon application of weights to the uterus, the amount and duration of the response being roughly related to the weight (see Fig. 1). Such slowing of the heart with increase of fetal blood pressure at the moment of application of external pressure may be called "pressure bradycardia."

Upon removal of the weights from the uterus, a second period of bradycardia occurs. It is added to the existing pressure bradycardia, if this still persists. Since this second bradycardia is associated with a sudden release of external pressure from the uterus and a consequent sudden diminution of fetal blood pressure, we may refer to such slowing of the heart as "release bradycardia" (see Fig. 1). When atropine is injected into a fetus exhibiting release bradycardia, the heart rate immediately increases (see Fig. 2). The release bradycardia is, accordingly, a reflex vagal inhibition of the heart. In this respect, it resembles the bradycardia that is associated with occlusion of the umbilical arteries or veins, as mentioned above.

The effect of graded weights upon the amniotic fluid and fetal blood pressures in one experiment is summarized in Figure 3. In this figure, the change from the initial amniotic fluid pressure is shown by the white bars and the change in fetal blood pressure is shown by the black bars. An increase in pressure is

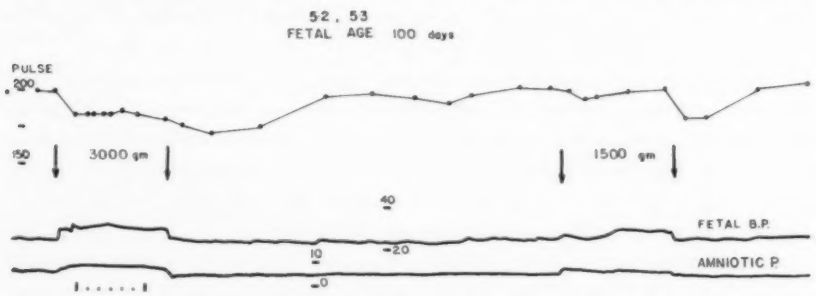

FIG. 1. Fetal blood pressure (mean) of the lamb *in utero*, and amniotic fluid pressure. Fetal heart rate curve above. Note (a) bradycardia on application of 3000-gm and 1500-gm weights, and (b) bradycardia on removal of weights. Pressure and release bradycardia. Minute intervals shown at bottom.

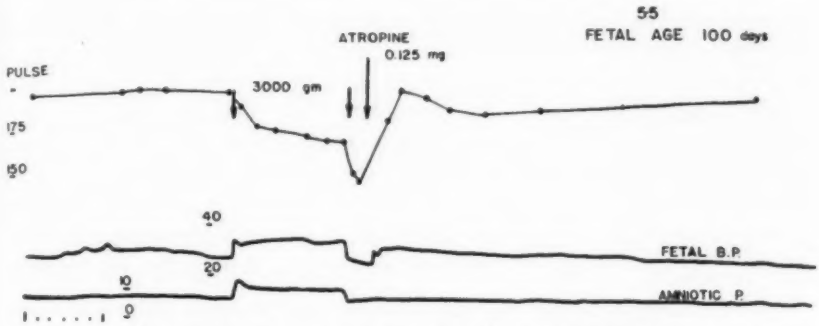

FIG. 2. Same as Figure 1, but atropine injected into fetus during release bradycardia caused an increase of fetal heart rate and blood pressure. Release of a vagal inhibition.

indicated above the line, a decrease below the resting or initial pressure level is shown below the line. It will be seen that the one-kilogram weight caused a slightly greater increase in amniotic fluid pressure than in fetal blood pressure. Blood pressure was variable after removal of the weight, while the intrauterine

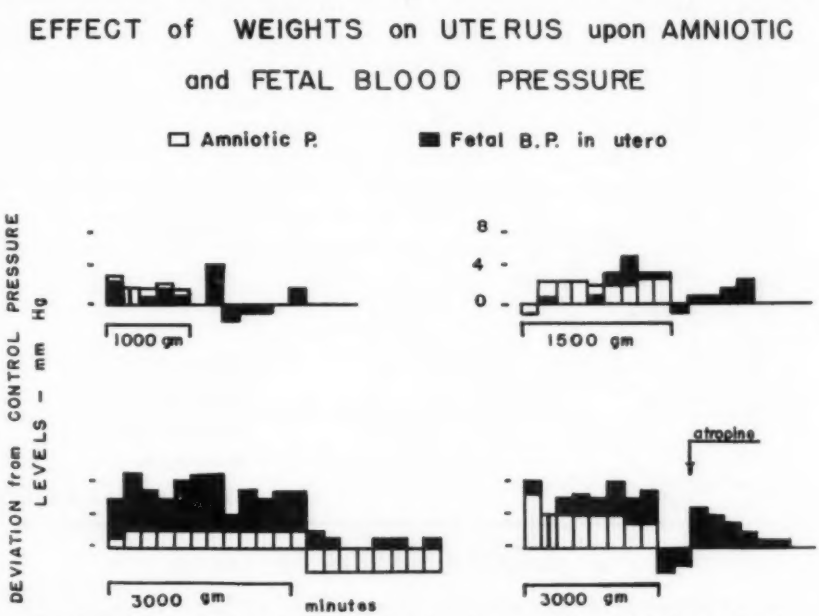

FIG. 3. Summary of effect of three weights placed on uterus in a single fetus. Clear bars, change of intrauterine blood pressure from normal; black bars, change in fetal blood pressure. Note the progressively greater change in fetal blood pressure with increased weight. See text for discussion.

pressure returned to normal. A 1500-gram weight has the same effect as the kilogram weight for the first five minutes. Then fetal blood pressure increased more than the amniotic fluid pressure for the next four minutes. Upon release of the pressure, there was a temporary fall of blood pressure below the initial pressure level. With a 3000-gram weight upon the uterus, the fetal blood pressure increased far more than the amniotic fluid pressure throughout the entire nine-minute period. On removal of the weight, there was a persistent decrease in amniotic fluid pressure, as if the uterus had been stretched excessively by the weight. Taken as a whole, the results of this experiment show that the response

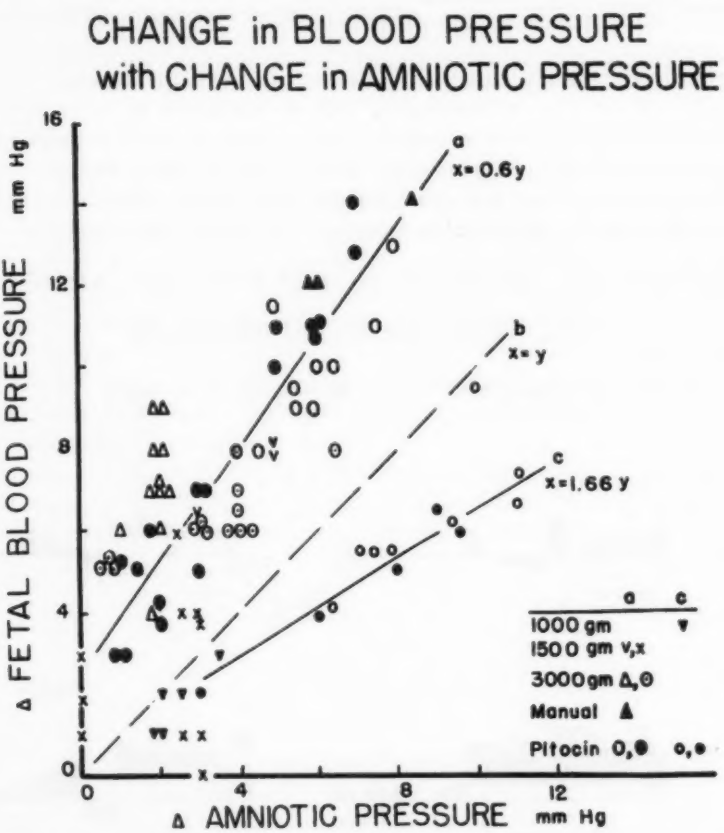

FIG. 4. Summary of effect of increasing intrauterine pressure three different ways (see legend) on fetal blood pressure. If the intrauterine pressure were simply additive on the fetal blood pressure, points would fall along line b (where $x = y$). Generally speaking, with stronger stimuli the blood pressure effect was greater than this (points along curve a (where $x = 0.6\ y$), and with weaker stimuli, along curve c (where $x = 1.66\ y$)).

of the fetal blood pressure to an increase in intrauterine pressure is greater when the weight is greater.

Blood pressure versus amniotic fluid pressure. Figure 4 summarizes our experience with respect to the amount of increase in fetal blood pressure and the different increments of amniotic fluid pressure. The graph includes the effects of increasing intrauterine pressure by the injection of Pitocin, as well as by putting weights on the uterus or manually pressing the uterus.

If the premise were true that, like a physical system, a given increment in amniotic fluid pressure causes an equal increase in fetal blood pressure, the points would fall along the broken line, b, where $x = y$. However, the fact is that some points fall along line a, where $x = 0.6y$, and the rise exceeds the environmental increment of pressure by about one-third. Other points fall along line c, where $x = 1.66y$, and the rise is about one-third less than would be expected on physical grounds alone.

The data show that physiological changes take place within the fetus in response to increase of intrauterine pressure which cause the fetal blood pressure to deviate from physical expectations alone. Although the blood pressure may rise either more or less than expected, a slowing of the fetal heart occurs in both cases. The bradycardia is, as we have seen, a vagal inhibition.

Injection of Pitocin. The injection of Pitocin in the ewe causes a sustained rise of intrauterine pressure. When one milliliter or less is injected slowly, the rise in fetal blood pressure tends to be less than the rise in intrauterine pressure. Even so, there is a profound pressure bradycardia, as shown by Figure 5. The slower heart beat continues for nearly half an hour for, as may be seen in Figure 5, the placing of a 1500-gram weight upon the uterus during the recovery period fails to elicit either a pressure or a release bradycardia. It may be that

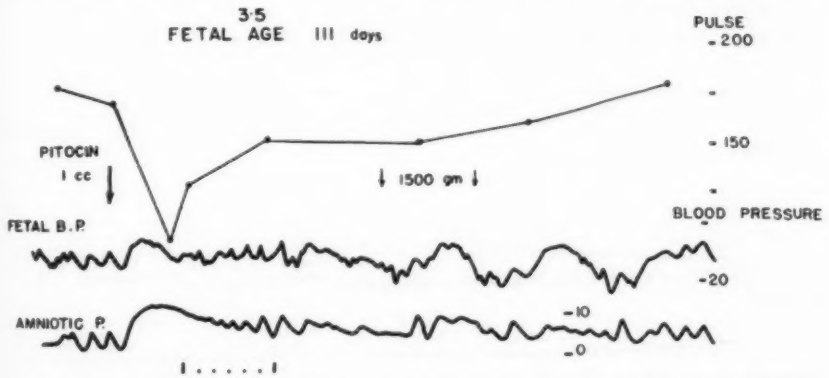

FIG. 5. Fetal bradycardia with increase of intrauterine pressure by injection of Pitocin into ewe. Note long-delayed recovery, and failure to demonstrate pressure or release bradycardia when 1500-gm weight was applied.

the vagal centers are already active and unresponsive to further stimulation, or it may be that the use of a 1500-gram weight is not now an adequate stimulus because the uterus is already somewhat firm as a result of the injection of Pitocin. In Figure 6, one may see that the injection of atropine into the fetus during the recovery period after injection of Pitocin causes a temporary increase in blood pressure, indicating the effect of a vagal release.

The nature of pressure bradycardia. Pressure bradycardia is, as noted, vagal in character, and it is associated with an increase in fetal blood pressure. Barcroft has explained this type of response in lamb fetuses on the basis of the action of a depressor nerve response resulting from the elevated aortic pressure, Barcroft (4). We have confirmed his observation that reflex bradycardia immediately follows occlusion of the umbilical arteries, Reynolds (2). However, the fact that bradycardia occurs when the umbilical veins only are occluded and there is a fall in blood pressure suggests that the vagal mechanism may be activated by other than sensory depressor receptors in the aorta. The release bradycardia is similar to the vein-occlusion effect, for vagal inhibition of the heart takes place with a fall of blood pressure.

It is possible that the common factor in both types of response is interference with the placental circulation, and that the vagal response in both instances is, therefore, the result of hypoxia in the fetus. Two types of experiment were carried out to test this idea. First, a ewe was given 100 per cent nitrogen to breathe to see if the fetal response to hypoxia is bradycardia resulting from vagal activity. This was done three times, once with one fetus, and twice with another. Second, while a ewe was given 100 per cent oxygen to breathe, the effect of increase of intrauterine pressure was tested to see if pressure and release bradycardia could be demonstrated when tissue oxygen pressure is high. This effect was observed twice.

The nitrogen experiment was carried out on a fetus of 120 days gestation

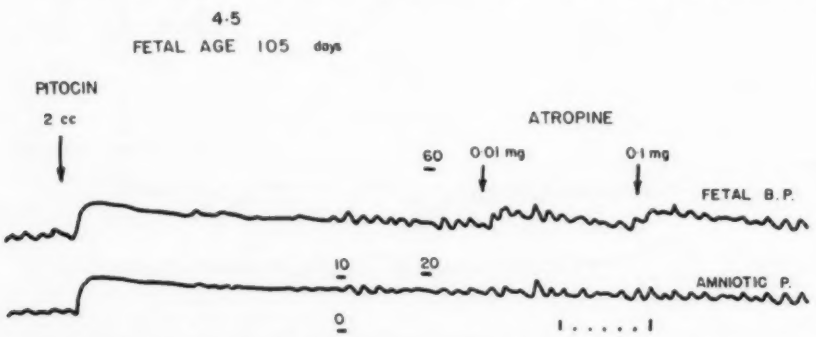

FIG. 6. Effect of atropine on fetal blood pressure after injection of Pitocin into ewe. Increase in blood pressure signifies release of a vagal inhibition (compare with Figure 2).

age. When the ewe was put on nitrogen, the typical adult type of response to asphyxia was obtained in the ewe. There was hyperpnea, tachycardia and elevation of blood pressure. This was followed by apnea, and a decline in pulse rate and blood pressure (see Fig. 6). In contrast to the response in the ewe, the response in the fetus was an immediate bradycardia with a slight fall in blood pressure. The bradycardia was vagal in origin for it was relieved at once when atropine was injected into the fetus two minutes before the ewe was restored to air (see Fig. 6). The atropine gave rise to an increase in pulse rate to 210 beats per minute which was unaffected by either application of a 5000-gram weight to the uterus or by its removal. Fetal blood pressure rose by an amount equal to the increase in intrauterine pressure.

The effect of acute hypoxia in the fetal lamb, accordingly, is to evoke a vagal response which mimics occlusion of the umbilical arteries with elevation of fetal blood pressure, or occlusion of the two umbilical veins with a fall in fetal blood pressure. The relation of these effects to application of weights to the uterus remains to be established, although it may be tentatively inferred as a working hypothesis that the pressure and release bradycardia are associated with an interference with the placental circulation, with resultant hypoxia of the fetus.

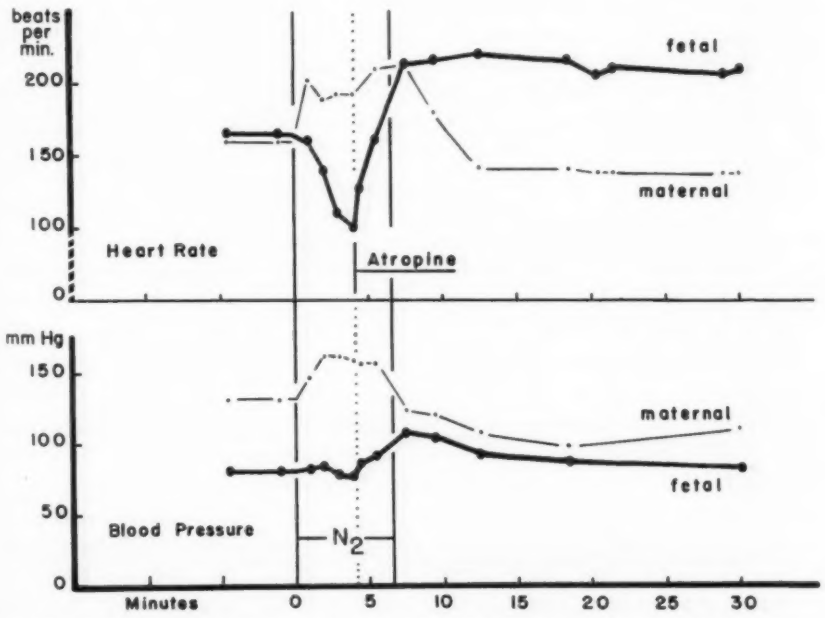

FIG. 7. Fetal bradycardia and maternal tachycardia by having the ewe breath nitrogen. Note release of fetal bradycardia upon injection of atropine into fetus.

The alternative experiment of administering oxygen to a ewe was carried out in two fetuses of 120 days gestation age. In each, the ewe was pre-oxygenated for twenty minutes before any test was made. By this time, if the same conditions exist in ewes as in the human, Armstrong (5), about 40 to 50 per cent of the nitrogen is eliminated from the body and the oxygen tension throughout the blood and tissues is raised from about 100 mm mercury to about 350 mm mercury.

The initial effect of O_2 was to cause a decrease of about twenty beats per minute in the heart rate of both the fetus and the ewe. There was no important effect on the blood pressure of ewe or fetus. When a weight of 3000 grams was placed upon the uterus, amniotic fluid pressure increased by 7 to 8 millimeters of mercury. A clear-cut bradycardia failed to occur. There was a slight but doubtfully significant decrease of ten beats per minute (see Fig. 7) at first but it did not clearly persist as is usually the case with the pressure bradycardia in response to a 3000-gram weight. There was no release bradycardia. Twelve minutes after cessation of oxygen, a pressure bradycardia still could not be produced but at 23 minutes there was a clear release bradycardia response which was inhibited by atropine (see Fig. 8).

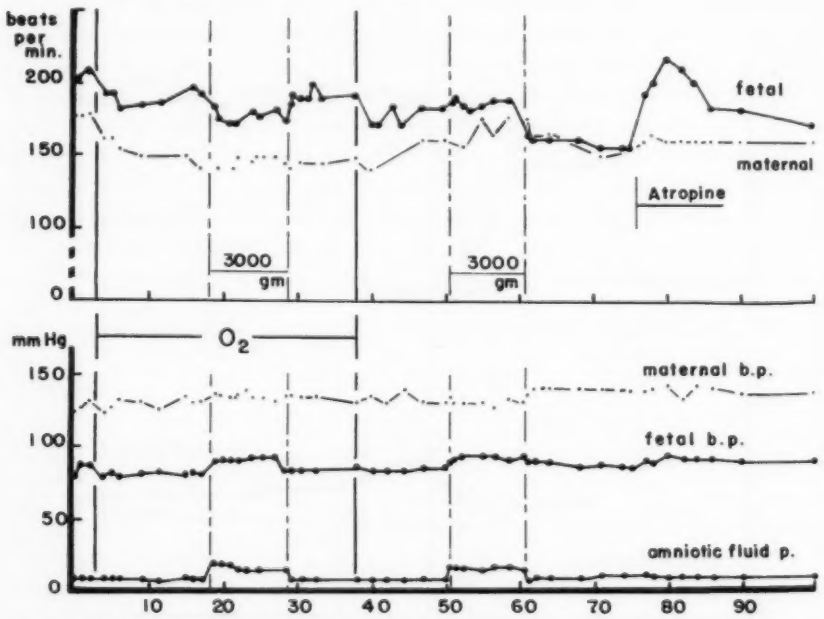

FIG. 8. Prevention of pressure and release bradycardia by putting the ewe on O_2 before application of weight to uterus. Note release bradycardia, and atropine release, twenty-three minutes after withdrawal of oxygen.

This experiment strongly suggests that the reflex pressure and release bradycardias which we see commonly with increase in intrauterine pressure are the result of central anoxia which serves to excite the vagal centers temporarily, so establishing vagal tone for a time. Such a conclusion is compatible, at least, with the fact that induced hypoxia simulates the pressure and release bradycardias; that oxygen protects the fetus against the effect of increased intrauterine pressure; and that the response to increased pressure within the uterus is simulated by interference with the placental circulation, whether by occlusion of the umbilical arteries or of the veins.

Compression of the fetal head and body. It is generally believed by obstetricians that slowing of the fetal heart occurs when forceps are applied to the fetal head and traction applied to the head for the purpose of aiding delivery. We repeatedly attempted to produce fetal cardiac slowing by applying local pressure on the fetal head, still *in utero*, and by applying traction on the head while holding the fetal body. All attempts met with failure, for we never produced an alteration in the fetal heart rate that we could detect.

SUMMARY

1. In 8 fetal lambs of 100–120 days gestation age, cardiovascular responses (blood pressure and heart rate) were studied in relation to increase in intrauterine pressure.

2. Intrauterine pressure was increased by rhythmic uterine contractions; by manual compression of the uterus, by placing weights of 1000 grams, 1500 grams, 3000 grams, and 5000 grams upon the uterus; and by injection of Pitocin into the ewe.

3. Rhythmic uterine contractions of low intensity caused an equal increase of blood pressure without a change in heart rate.

4. When intrauterine pressure was increased by the application of more weights, an increase in fetal blood pressure occurred, but this increase was either substantially more or less than the increase in amniotic fluid pressure. There was invariably a fetal bradycardia which could be inhibited by atropine. Upon removal of the weights, a second bradycardia response, vagal in character, occurred.

5. Pitocin injected into the ewe gave a fetal response with strong uterine contraction similar to the response incurred by the application of weights.

6. Simple hypoxia (inhalation of N_2 by the ewe) gave a fetal bradycardia, vagal in character, but a tachycardia in the ewe with an elevation of blood pressure. Inhalation of oxygen prevented fetal bradycardia with otherwise adequate stimuli.

7. The hypothesis is developed that fetal bradycardia upon marked increase of intrauterine pressure is the result of acute fetal hypoxia associated with interference with the placental circulation.

8. Pressure and traction on the fetal head had no observable effect upon fetal heart rate or blood pressure.

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A COMPARISON OF THE METABOLISM OF ETHANOL AND TRICHLOROETHANOL[1, 2]

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There seems little doubt that the oxidation of ethanol proceeds in a stepwise manner, first to acetaldehyde and then to acetic acid or active acetyl. Tetraethylthiuramdisulfide (disulfiram) and cyanamide are stated to inhibit the oxidation of acetaldehyde and influence the primary step in ethanol oxidation only when given in much larger doses (1).

When either trichloroethanol (TCE) or chloral hydrate is given to dog or man a portion is oxidized to trichloroacetic acid (TCA). The production of TCA from chloral hydrate begins promptly before an oral dose is entirely absorbed and is apparently completed within the first hour. However, the production of TCA from TCE proceeds more slowly and may continue for 24 to 48 hours (2).

The present report is concerned with certain aspects of the intermediary metabolism of ethanol and trichloroethanol in the dog, the effect of ethanol and acetaldehyde on the oxidation of TCE and chloral hydrate, and a comparison of the influence of disulfiram and cyanamide on the metabolism of ethanol, TCE, acetaldehyde and chloral hydrate. These *in vivo* studies are supplemented by certain *in vitro* observations.

METHODS

Trained female dogs in the postabsorptive state were utilized. Blood was drawn from a vein. When administered orally, ethanol, TCE[4] and chloral hydrate were given in 20 ml of water per kilogram of body weight. When given intravenously, ethanol, TCE, acetaldehyde and chloral hydrate were dissolved in isotonic saline. The acetaldehyde was redistilled immediately prior to use. Disulfiram,[5] 300 mg per kilogram, was administered

[1] This investigation has been aided by a grant from the U. S. Public Health Service.

[2] A preliminary report of some of these experiments was presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, September 6–8, 1954. The statement that disulfiram does not affect the conversion of chloral hydrate to TCA was later found to be incorrect (J. Pharmacol. and Exper. Therap., 1955, **113:** 46).

[3] E. R. Squibb Fellow in Pharmacology and Experimental Therapeutics.

[4] We wish to thank Mr. W. A. Lott of E. R. Squibb and Sons for supplying us with trichloroethanol used in this investigation.

[5] We wish to thank the Ayerst Laboratories Incorporated for the disulfiram used in this investigation.

orally in gelatin capsules 18 to 22 hours prior to an experiment. Calcium cyanamide,[6] 60 mg per kilogram, also in capsules, was given 2 to 4 hours before an experiment. Both inhibitors were given with 20 ml of water per kilogram.

TCE, chloral hydrate and TCA were determined as previously described (2). When plasma containing relatively high concentrations of TCE was to be analyzed for chloral hydrate or TCA, the TCE in the filtrate was first extracted with 5 volumes of benzene. Ethanol was determined using alcohol dehydrogenase as described by Marshall and Fritz (3). Acetaldehyde was estimated by the method of Stotz (4).

RESULTS

The effect of disulfiram and cyanamide on the oxidation of TCE

When TCE, 54 mg per kilogram, is given to a dog TCA regularly appears in the plasma. The results of a typical experiment are shown in Figure 1. If disulfiram or cyanamide is administered prior to TCE no TCA (i.e. less than 0.1 mg per 100 ml) can be detected in the plasma. Consistent results were obtained in 10 paired experiments with and without disulfiram and 6 paired experiments with and without cyanamide. In one instance when a similar dose of calcium cyanamide was given 17 instead of 2 to 4 hours prior to the administration of TCE, TCA did appear in the plasma in less than the normally expected concentrations. In these inhibitor experiments, chloral hydrate was never detected in the plasma.

The effect of disulfiram and cyanamide on the oxidation of chloral hydrate

In contrast to the complete inhibition of the oxidation of TCE to TCA, the inhibitors did not entirely block the appearance of TCA in the plasma following

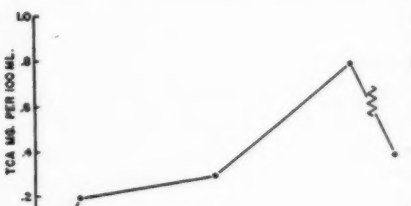

FIG. 1

FIG. 2

FIG. 1. Plasma concentrations of TCA following an oral dose of TCE, 54 mg per kilogram. The prior administration of disulfiram or cyanamide and the repeated administration of ethanol precluded the appearance of TCA in the plasma. (Dog. Liz in each instance)

FIG. 2. Plasma concentrations of TCA following an oral dose of chloral hydrate, 15 mg per kilogram. The effect of premedication with cyanamide (○---○) is contrasted with a control experiment (●—●). (Dog Liz both instances)

[6] We wish to thank Dr. John T. Litchfield, Jr. of the American Cyanamid Company for the calcium cyanamid used in this investigation.

the oral administration of chloral hydrate. In 10 paired experiments (5 with disulfiram) the effect of the inhibitor on the oxidation of chloral hydrate 7, 15 or 60 mg per kilogram given orally was noted. Examination of our data shows that in each instance and at each dose level of chloral hydrate, the amount of TCA which accumulates in the plasma following the administration of either inhibitor is definitely less than in the paired control. Figures 2, 3 and 4 illustrate these observations.

Further observations were made concerning the persistence in the plasma of intravenously administered chloral hydrate following premedication with an inhibitor. Ten paired experiments (5 with cyanamide) were performed in which chloral hydrate 15, 30 or 60 mg per kilogram, was infused at a constant rate over a 3-minute period and blood samples withdrawn 1, 3, 6, 9, 12 and 20 minutes following completion of the infusion. In the forenoon chloral hydrate was given without premedication. Forty to 50 minutes following the completion of the morning experiment cyanamide was administered and chloral hydrate again infused 3½ hours later. Appropriate control experiments showed that any residua from the forenoon procedure in no way interfered with the clearance of chloral hydrate from the plasma or the accumulation of TCA subsequent to the second injection of chloral hydrate. Two additional experiments using cyanamide were run on 2 standardized dogs at a time when their plasma was free of TCA. The results in no way differed from those obtained in the above described experimental sequence.

Although the magnitude of the effect of cyanamide on the clearance of chloral hydrate from the plasma varied in different experiments, the results were qualitatively consistent. In each instance the concentrations of chloral

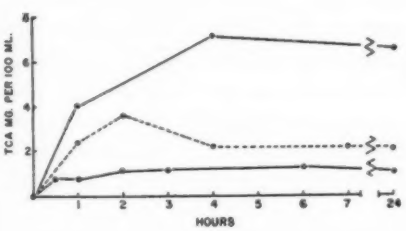

FIG. 3

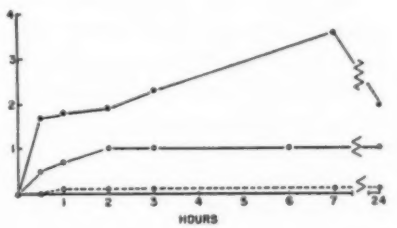

FIG. 4

FIG. 3. Plasma concentrations of TCA following an oral dose of chloral hydrate, 60 mg per kilogram. The effect of premedication with disulfiram (○---○) and repeated administration of ethanol (○ — ○) is contrasted with a control experiment (● — ●) (Dog Tana in each instance)

FIG. 4. Plasma concentrations of TCA following an oral dose of chloral hydrate, 60 mg per kilogram. The effect of premedication with cyanamide (○---○) and repeated administration of ethanol (○ — ○) is contrasted with a control experiment (● — ●). (Dog Ches in each instance)

hydrate were greater after cyanamide had been given. In 6 of the 7 experiments with the inhibitor detectable amounts of chloral hydrate persisted in the plasma longer than in their paired controls. Figure 5 illustrates one of these experiments.

The effect of cyanamide on the accumulation of TCA in the plasma in these experiments was not definite. In 3 of 7 experiments with the inhibitor the plasma concentrations of TCA were significantly less than their control values, whereas the concentrations in the 4 other trials did not differ from their controls. This is somewhat in contrast to the inhibition of the oxidation of orally administered chloral hydrate previously noted.

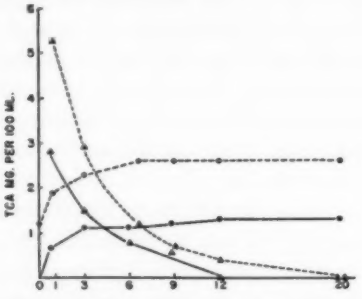

FIG. 5. Plasma concentrations of chloral hydrate (▲ — ▲) and TCA (● — ●) following a dose of chloral hydrate, 30 mg per kilogram, injected intravenously at a constant rate over a 3-minute period. Included are the concentrations of chloral hydrate (△---△) and TCA (○---○) in a similar experiment in the same dog premedicated with cyanamide.

The effect of disulfiram and cyanamide on the metabolism of ethanol

Disulfiram and cyanamide have been shown to affect the metabolism of ethanol primarily by inhibiting the oxidation of acetaldehyde. In experiments with disulfiram, which in the main have been performed on rabbits, no decrease occurred in the rate of disappearance of ethanol from the blood except when large doses of the inhibitor were given. In addition, it was shown that the concentrations of acetaldehyde in the blood were increased by administration of disulfiram, but that no decrease was noted in the clearance of acetaldehyde from the blood (1).

The dog apparently differs from the rabbit and man in that no marked increase is noted in the blood concentration of acetaldehyde following the administration of ethanol. When 0.6 gm per kilogram of ethanol was given intravenously to dogs which had previously received disulfiram or cyanamide no effect was seen on the rate of clearance of ethanol from the blood. There was, however, a marked increase in the blood concentrations of acetaldehyde over the control values. (See Figs. 6 and 7.)

Eight paired experiments (4 with an inhibitor) were performed to demonstrate the effect of disulfiram and cyanamide on the clearance from the blood of acetaldehyde, 4 ml of a 1 per cent solution (40 mg) per kilogram, infused intravenously at a constant rate over a 3-minute period. These experiments were performed in a similar manner to those reported by Westerfeld *et al.* (5) and those previously described concerning intravenously administered chloral hydrate. Control observations on these dogs similar to those used with chloral hydrate demonstrated no objection to our procedure.

Following the infusion of acetaldehyde, ethanol appears in the plasma in concentrations ranging from 0.4 to 0.9 mg per 100 ml (the "normal" ethanol of dog blood with our method is 0.0 to 0.1 mg per 100 ml. No increase in this "normal" plasma ethanol is noted when either disulfiram or cyanamide is given alone.) When acetaldehyde is given to a dog premedicated with an inhibitor, the plasma concentrations of ethanol found are increased, ranging up to 2 mg per 100 ml. Further, the blood concentrations of acetaldehyde are greater and this substance is cleared from the blood less rapidly than in the control experiment. Typical results are illustrated by Figures 8 and 9.

The effect of ethanol and acetaldehyde on the oxidation of TCE

When TCE, 54 mg per kilogram, is given intravenously or orally to a dog which has previously received ethanol, 0.6 gm per kilogram, no TCA appears in the plasma. The dose of ethanol was repeated during the procedure to maintain a plasma concentration above 20 mg per 100 ml. One of two experi-

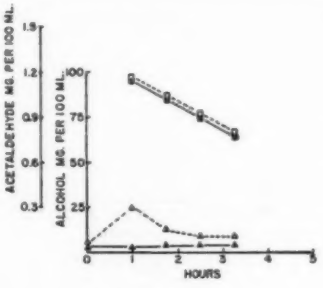

FIG. 6

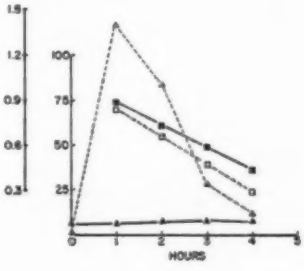

FIG. 7

FIG. 6. Plasma concentrations of ethanol (■ — ■) and blood concentrations of acetaldehyde (▲ — ▲) following an intravenous dose of ethanol, 0.6 mg per kilogram. Included are the concentrations of ethanol (□---□) and acetaldehyde (△---△) obtained in a similar experiment in the same dog premedicated with disulfiram.

FIG. 7. Plasma concentrations of ethanol (■ — ■) and blood concentrations of acetaldehyde (▲ — ▲) following an intravenous dose of ethanol, 0.6 gm per kilogram. Included are the concentrations of ethanol (□---□) and acetaldehyde (△---△) obtained in a similar experiment in the same dog premedicated with cyanamide.

ments is shown in Figure 1. In one dog given TCE, 162 mg per kilogram, in a similar experiment no TCA was observed in the plasma during the subsequent 2½ hours. Further, if acetaldehyde is infused intravenously to maintain blood concentrations ranging from 1.5 to 2.0 mg per 100 ml and TCE, 54 or 225 mg per kilogram, is given to the animal no TCA appears in the plasma during the infusion period which extended to 4 hours in our 2 experiments.

The effect of ethanol and acetaldehyde on the oxidation of chloral hydrate

The repeated administration of ethanol, sufficient to maintain a plasma level above 20 mg per 100 ml consistently inhibits the oxidation of chloral hydrate to TCA. Results typical of 6 experiments are shown in Figures 3 and 4. Acetaldehyde infused continuously intravenously is also a potent inhibitor of chloral hydrate oxidation when the latter is given orally. (See Fig. 10.) If, as in previously described experiments, chloral hydrate is given intravenously over a 3-minute period while the blood concentration of acetaldehyde is maintained from 1 to 2 mg per 100 ml, the concentrations of chloral hydrate are higher and persist longer in the plasma than in paired control experiments. (See Fig. 11.) Further, the production of TCA is consistently decreased under these circumstances.

The oxidation of TCE and chloral hydrate in vitro

When TCE or chloral hydrate is incubated at 37.5° C with dog or rat liver slices the production of TCA occurs. In our experiments fresh livers were

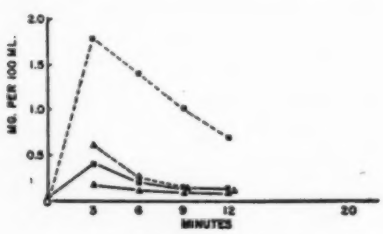

FIG. 8

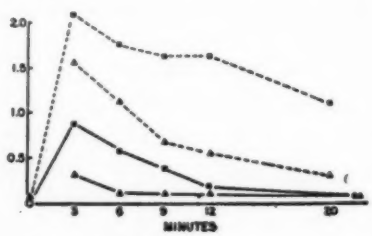

FIG. 9

FIG. 8. Plasma concentrations of ethanol (■ — ■) and blood concentrations of acetaldehyde (▲ — ▲) following a dose of acetaldehyde, 40 mg per kilogram, injected intravenously at a constant rate over a 3-minute period. Compare the concentrations of ethanol (□ --- □) and acetaldehyde (△ --- △) in a similar experiment in the same dog premedicated with disulfiram.

FIG. 9. Plasma concentrations of ethanol (■ — ■) and blood concentrations of acetaldehyde (▲ — ▲) following a dose of acetaldehyde, 40 mg per kilogram, injected intravenously at a constant rate over a 3-minute period. Compare the concentrations of ethanol (□ --- □) and acetaldehyde (△ --- △) in a similar experiment in the same dog premedicated with cyanamide.

sliced with a Stadie-Riggs apparatus and 500–700 mg of tissue (weighed after slicing) were placed in a stoppered vessel containing 3 ml of Krebs-Henseleit buffer in which was dissolved dextrose, 6 mg, and TCE or chloral hydrate, 1×10^{-6} moles. The gas phase, oxygen, 95 per cent and CO_2, 5 per cent was maintained continuously. The reaction vessels were agitated in a water bath at 37.5° C for 1 to 3 hours.

In the experiments with TCE, approximately 4×10^{-8} moles of TCA were produced following a 1-hour incubation and 0.8 to 1.2×10^{-7} moles of TCA had accumulated after a 3-hour incubation. When similarly reaction mixtures containing slices from the same livers were incubated concomitantly in the presence of disulfiram, 10 mg, no TCA (less than 2×10^{-8} moles) was observed in the flask.

In the experiments with chloral hydrate, the maximum amount observed of TCA (0.7 to 1.2×10^{-7} moles) was consistently produced during a 1-hour incubation. If disulfiram, 10 mg, was added to the reaction mixture no effect was noted in the accumulation of TCA. (Under these circumstances we were unable to lower the chloral hydrate concentration and increase the disulfiram concentration.)

Chloral hydrate is also oxidized to TCA in a buffered solution containing dog and rat liver homogenate. A lyophilized aqueous extract of dog liver acetone powder contains a higher concentration of the chloral oxidizing enzyme.

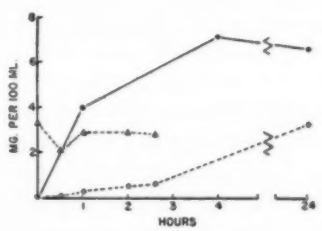

FIG. 10

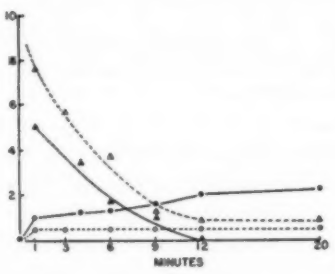

FIG. 11

FIG. 10. Plasma concentrations of TCA (● — ●) following an oral dose of chloral hydrate, 60 mg per kilogram. In a similar experiment in the same dog, the effect of 5 per cent acetaldehyde infused intravenously to maintain a blood concentration (△ --- △) over a 2.5 hour period is shown on the appearance of TCA (○ --- ○) in the plasma.

FIG. 11. Plasma concentrations of chloral hydrate (▲ — ▲) and TCA (● — ●) following a dose of chloral hydrate, 60 mg per kilogram, injected intravenously, at a constant rate over a 3-minute period. In a similar experiment in the same dog, the effect of 5 per cent acetaldehyde administered intravenously to maintain a blood concentration from 1–2 mg per 100 ml during the procedure is shown on the clearance of chloral hydrate (△ --- △) from the plasma and the appearance of TCA (○ --- ○) in the plasma.

The inhibitory effect of disulfiram or cyanamide on the production of TCA could not be demonstrated with these preparations.

The reduction of chloral hydrate by horse liver alcohol dehydrogenase

The reduction of chloral hydrate can be catalyzed *in vitro* by crystalline horse liver alcohol dehydrogenase[7] with reduced diphosphopyridine nucleotide (DPNH) being the reductant. The reaction will proceed in a 0.1 M phosphate buffer, pH 7.2, at room temperature (23–26° C) and can be followed by observing the decreasing concentration of reduced DPN with a Beckman spectrophotometer. Our experiments were performed in 1 cm square cuvettes containing 3.0 ml phosphate buffer in which was dissolved alcohol dehydrogenase and DPNH,[8] 4.4×10^{-6} moles. The reaction was initiated by the addition of chloral hydrate and the readings at 340 mu were noted every 15

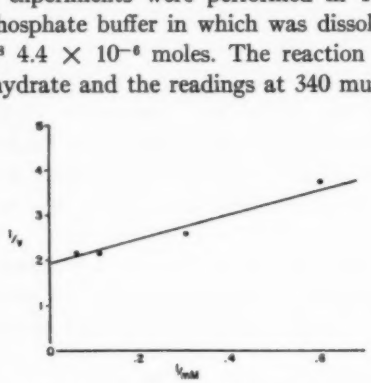

FIG. 12. The reduction of chloral hydrate by horse liver alcohol dehydrogenase. The relationship of the substrate concentration and initial velocity is plotted according to Lineweaver and Burk.

seconds. Figure 12 illustrates the relationship between the initial velocity (decrease in optical density during the first minute) and the substrate concentration plotted according to the method of Lineweaver and Burk (6). The Michaelis constant for chloral hydrate thus calculated is 1.4×10^{-3} M. This compares with a Km of 1.2×10^{-4} M for acetaldehyde obtained by Theorell and Bonnichsen (7) with the same enzyme.

The oxidation of TCE was found not to proceed in the presence of liver or yeast alcohol dehydrogenase and DPN in various buffers and at pH 7.2 to 10. When TCE was added to initiate the reaction, the solution became cloudy and no reduction of DPN could be noted.

[7] We wish to thank Dr. Nathan O. Kaplan for horse liver alcohol dehydrogenase used in this investigation.

[8] Recent batches of DPN and DPNH obtained from Sigma appeared to contain alcohol. This could be removed by dissolving the compounds in water and lyophilizing.

DISCUSSION

Existing evidence together with the observations made during the present investigation would appear to indicate that ethanol and trichloroethanol share common pathways of metabolism. It is well established that ethanol is first oxidized to acetaldehyde by alcohol dehydrogenase and that acetaldehyde is then oxidized to acetic acid probably by aldehyde dehydrogenase. The inhibitors (disulfiram and cyanamide) block the oxidation of acetaldehyde but do not affect the rate of oxidation of ethanol. The acetaldehyde concentration in the blood rises and then apparently its oxidation proceeds to acetic acid. On the other hand, the oxidation of TCE to TCA is completely prevented and the oxidation of chloral hydrate to TCA is inhibited but not completely prevented by administration of the inhibitors.

The data presented in the present report would seem to indicate that the site of action of the inhibitors in the TCE–CH–TCA system is the same as that in the ethanol-acetaldehyde-acetate system. The observations which support the above interpretation are as follows. Horse liver alcohol dehydrogenase catalyzes the reduction of chloral hydrate to TCE although we have been unable to obtain catalysis by this enzyme of the reverse reaction. The inhibitors act qualitatively in a similar manner on the rate of disappearance of injected chloral hydrate and acetaldehyde, raising the concentrations of these substances and prolonging their presence in the blood. The oxidation of TCE to TCA can be completely prevented by the previous and concomitant administration of either ethanol or acetaldehyde. Although chloral hydrate has never been detected in the plasma of our dogs either after administration of TCE alone or after an inhibitor, it would seem logical to assume that chloral hydrate is an intermediate in view of the ability of acetaldehyde to completely block the oxidation of TCE to TCA. Apparently ethanol and acetaldehyde have a greater affinity for the enzymes catalyzing the oxidation of TCE and chloral hydrate.

The apparent differences in the effect of disulfiram and cyanamide on the TCE and ethanol systems might be explained as follows. When TCE is given to a dog most of the dose is quickly conjugated with glucuronic acid and only a small portion is oxidized. On the other hand, nearly all of a given dose of ethanol is oxidized to acetaldehyde. When chloral hydrate is administered, a large amount is rapidly reduced to TCE (and quickly conjugated) and a smaller amount is oxidized to TCA. This is in contrast to the very small production of ethanol and presumably rapid oxidation of the major portion of injected acetaldehyde. Apparently liver alcohol dehydrogenase is not the only enzyme capable of reducing chloral hydrate since Butler (8) found that blood and all tissues studied were capable of forming TCE from chloral hydrate.

Thus the administration of either disulfiram or cyanamide causes a marked increase in the concentration of acetaldehyde in the blood when ethanol is given, but no chloral hydrate can be detected when TCE is given after an inhibitor. The oxidation of TCE to TCA, then, is prevented. When chloral hydrate is administered after premedication with an inhibitor, its oxidation to TCA will proceed only while the concentration of chloral hydrate is sufficiently high to overcome the inhibition. The rapid reduction of chloral hydrate prevents the maintenance of high concentrations, hence less chloral hydrate is oxidized following the administration of an inhibitor than normally would be expected.

SUMMARY

A study has been made of the intermediary metabolism of ethanol and trichloroethanol in the dog. Our observations would appear to indicate that ethanol and trichloroethanol share common pathways of metabolism.

ACKNOWLEDGMENT

We wish to thank Sara Ann Verplanck for technical assistance.

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STUDIES ON COPPER METABOLISM

XVIII. Skeletal Changes Associated with Copper Deficiency in Swine*

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During the course of observations on copper deficiency in swine which have been reported elsewhere (1) certain manifestations appeared which pointed to involvement of the skeleton. These were not wholly unexpected since deformaties in the extremities had been described before in swine (2), and others (3, 4) have reported skeletal defects in cattle and lambs. More recently, Baxter and Van Wyk (5) have presented the clinical manifestations of copper deficiency in dogs and, in association with them, one of us (R. H. F., Jr.) has studied the histological changes in the bones (6). The conclusion reached was that copper deficiency appears to lead to interference with osteoblastic activity while destructive sequences go on in normal fashion. So, too, growth of the cartilage appears to occur in normal fashion, although there is reduced deposition of bone on the calcified cartilagenous matrix.

The present report deals with an examination of the bones of a series of swine deficient in copper. The changes found appear similar to those observed in the dog, and evidence is presented which places on a firmer basis the hypothesis that copper deficiency seriously affects osteoblastic activity.

MATERIALS AND METHODS

Four swine of the Chester-White breed were used in this study. The pigs were from a single litter. They were reared on wooden floors until 9 days of age, at which time they were separated from the sow and placed in individual galvanized iron pens with galvanized iron screen floors and stainless steel feeding troughs.

All of the animals were fed a basal diet (1) which consisted of a standard commercial brand§ of canned evaporated milk, diluted 1:1 with tap water. The tap water, prior to its addition to the milk, was allowed to stand for 24

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§ Carnation Company homogenized evaporated milk (25 U.S.P. units of vitamin D_3 added to each fluid ounce).

hours in a five gallon glass container to which 10 ml. of a 0.36 per cent solution of sodium sulfide were added. The diet was fed to each pig in an amount of 230 ml. (152 calories) per kg. of body weight per day. All lots of milk were analyzed for copper and were found to contain from 0.10 to 0.18 mg. of copper per liter of undiluted milk. Reduced iron*, spectroscopically free of copper, was dissolved in a minimum quantity of hydrochloric acid, diluted and added daily to the diet of all of the animals in such an amount that each animal received 30 mg. of iron per kg. of body weight.

All of the animals received a mineral (5) and a vitamin supplement. The composition of the mineral supplement was as follows (gm): sodium chloride (NaCl), 107; potassium chloride (KCl), 120; primary (monobasic) potassium phosphate (KH_2PO_4), 310; calcium phosphate ($Ca_3(PO_4)_2$), 150; calcium carbonate ($CaCO_3$), 210; magnesium ($MgSO_4 \cdot 7H_2O$), 192; iron ($FeCl_2 \cdot 4H_2O$), 15.6; manganese chloride ($MnCl_2 \cdot 4H_2O$), 0.3; aluminum sulfate ($Al(SO_4)_3 \cdot 18H_2O$), 0.1; sodium fluoride (NaF), 0.5; potassium iodide (KI), 0.5; zinc sulfate ($ZnSO_4 \cdot 7H_2O$), 0.3; and cobaltous nitrate ($Co(NO_3)_2 \cdot 6H_2O$), 0.3. This supplement was added to the diluted milk in an amount of 0.5 gm. per 100 ml. The vitamin supplement was as follows (mg./kg. of body weight/day): thiamin hydrochloride, 0.25; riboflavin, 0.12; nicotinic acid, 1.20; pyridoxine hydrochloride, 0.20; calcium pantothenate, 0.50; inositol, 0.20; paraaminobenzoic acid, 0.10; biotin, 0.10; pteroylglutamic acid, 0.10; and cobalamine, 0.10. The vitamins were placed in capsules and administered three times a week. In addition, all animals were given 10 mg. of choline chloride per kg. of body weight per day.

Pigs 14-48 and 14-49 served as controls and were given 0.5 mg. of copper per kg. of body weight daily. This was added to the diet in the form of an aqueous solution of copper sulfate†. No copper was given to pigs 14-46 and 14-47.

The animals were sacrificed between the ages of 136 and 160 days. At autopsy blocks were taken of the heart, liver, spleen, kidneys, and bones. All were fixed in formalin and Zenker-formol. Sections were made of the soft tissues as well as costochondral junctions, epiphysial lines of long bones and cross sections of diaphyses.

CLINICAL DESCRIPTION OF THE ANIMALS

By the time pigs 14-46 and 14-47 had been consuming the copper deficient diet for about 100 days, gross deformities of the legs were noted. These consisted of lateral bowing of the front legs and an increase in the posterior curva-

* Carbonyl iron powder, grade Rx, Centara Products, General Analine and Film Corporation, 444 Madison Ave., New York 22, N. Y.

† C. P. Baker's analyzed $CuSO_4 \cdot 5H_2O$.

ture of the hind legs. These deformities developed insidiously and were progressive. Similar gross skeletal defects were noted in copper deficient swine by Teague and Carpenter (2). In addition, fibrous knobs developed on the extremities, apparently at the points on which the animals lay. These became ulcerated. No bony abnormalities were noted in the control pigs (14-48 and 14-49).

Both of the copper deficient animals developed hematologic alterations characteristic of this deficiency in swine (1).

MICROSCOPIC FINDINGS

Sections of the heart, liver, spleen and kidneys showed no abnormality.

Description of the skeletal tissues can best be divided into two parts: cartilage-shaft junction and cortex (diaphysis).

Cartilage-shaft Junction: In the epiphysial cartilages of the controls (Nos. 48 and 49), the cells are proliferating in normal fashion; the zone of hypertrophic cells is of usual width. Blue staining material in the intercellular matrix of these cells indicates that the deposition of inorganic materials is taking place in normal fashion. In the upper metaphysis these spicules are prominent as a lattice-work of thin structures devoid of bone (Fig. 1). However, very soon osteoblasts are found approximated to these spicules of calcified cartilage matrix; pink staining bone appears on them in greater quantities as one proceeds deeper into the metaphysis. Ordinarily in swine there is a good deal of cartilage matrix incorporated in bone deep in the shaft. Such was seen in these animals. So, too, one usually finds numerous osteoclasts. These cells are also prominent in the sections and only indicate rapid growth. The osteoblasts, which are numerous, have abundant cytoplasm (Fig. 3). No particular changes are noted in the marrow elements.

In the two copper deficient animals the appearance of the cartilage resembles that of the preceding group. So, too, the deposition of inorganic materials in the cartilage matrix appears to be taking place in normal fashion. Here, however, similarity ceases between the two groups. The spicules of calcified cartilage matrix are for the most part bare of any enveloping bone (Fig. 2). They also appear to be increased in amount: i.e., are not being destroyed. There are very few cells that could be said to resemble ostoblasts. To be sure, there are flattened, elongated cells with nuclei similar to those of osteoblasts; they lack the basophilia of the cytoplasm and have more a fibroblastic than osteoblastic appearance (Fig. 4). The difference between the two groups is striking. No fractures are found at the cartilage-shaft junction of the rib. In areas in the long bones one is impressed by the inordinately excessive amounts of cartilage matrix devoid of bone (Fig. 5). Below such areas fracture patterns are clear enough. These consist of masses of calcified matrix in disarray bound together by irregular masses of bone (Fig. 6).

In summary, the differences in the two groups consist of a marked diminution of osteoblastic activity in the deficient animals. This is brought out by the great paucity of bone formation on the calcified cartilagenous matrix and by the persistence of the latter. Fractures of the calcified cartilage matrix "lattice" occur, and one finds evidence of their healing.

Cortex: Cross sections of the diaphyses of the long bones reveal a normal structure. The Haversian canals are small, though numerous (Fig. 7). There is more activity beneath the endosteum and periosteum as evidenced by their trabecular formation. In contrast the copper deficient swine show cortices which are thinner than those of the controls (Fig. 8).

DISCUSSION

Skeletal changes have been described in association with copper deficiency in several species (1–6), though in only one, the dog (6), have microscopic observations been reported. In the discussion of the changes in dogs it was suggested that copper deficiency may express itself by interfering in some way with normal osteoblastic activity. This suggestion was made because there was a paucity of bone formed on the spicules of calcified cartilage matrix in the metaphysis and because there was persistence of the cartilage matrix. An important consideration was pointed out: that the growth of cartilage was not interfered with. Hence, a complete dissociation between chondroblastic and osteoblastic activities was present; this indicated a specific disturbance in the osteoblast only, not the usual cessation of both activities which one observes as a result of inanition or lack of other essential nutrients, save ascorbic acid. The changes in the diaphyses of the dogs were interpreted as resulting from increased destructive activity in the face of decreased formation by the osteoblasts. Although it was thought that the destruction might be excessive, osteolytic sequences could just as well be going on at a normal level which, coupled with decreased formation, would ultimately lead to a thinner shaft.

It is, therefore, reassuring to one of us (R.H.F., Jr.) to find a picture in the swine similar to that which has been described above. The morphological evidence of decreased osteoblastic activity in another species would seem to furnish incontrovertible evidence that the functions of the osteoblasts are severely affected as a result of copper deficiency. In contrast, the growth of the cartilage cells seems hardly touched by the lack of this element. The failure of strengthening bone to be deposited on the thin spicules of calcified cartilage matrix leads to a weakened area which may be subject to fracture as a result of normal stress and strain. The evidence of previous fractures is seen in the metaphasis as masses of calcified matrix showing an abnormal architecture and surrounded by an excess of bone. The presence of some bone, of course, indicates that there is not complete cessation of osteoblastic activity. However, the fractures themselves may stimulate the osteoblast to increased activity.

The cortices, i.e., the diaphysial portions of the bone, are a little thinner than in the controls. Here it is likely that bone production is lagging over normal destruction. This observation is inferred more on the basis of what has already been seen at the cartilage shaft junction than for any other reason.

As was pointed out above, in the great majority of deficiency states in growing animals, particularly those due to a lack of a single essential nutrient in the diet, both chondroblastic and osteoblastic activities cease together. Other tissues usually stop growing, too. Until the observations on copper deficiency in the dog were described, the only instance of a deficiency state in which a dissociation of the activities of cartilage and bone cells had been reported was that due to ascorbic acid. The changes noted in the dog were likened to those observed in scurvy; so, too, the alterations observed in these swine are also more than reminiscent of scurvy. At least copper and ascorbic acid would seem to have a unique property in common: to interfere specifically with the functional activity of the osteoblast while not affecting in the least the integrity of cartilage cells.

SUMMARY

Microscopic examination of the bones of copper deficient swine indicates that there is marked reduction in osteoblastic activity as evidenced by a failure of bone to be deposited on the calcified cartilage matrix; nor is the latter removed. In contrast, growth of cartilage is not affected. It would appear that thinning of the cortex is due to decreased osteoblastic activity as well.

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PLATE I

FIG. 1. Cartilage-shaft junction of control animal (No. 49) to show orderly arrangement of spicules of calcified cartilage matrix upon which bone is being deposited by rows of osteoblasts.

FIG. 2. Cartilage-shaft junction of copper-deficient animal (No. 47) to show "lattice" of calcified cartilage matrix in excess of that seen in the control. Note also diminished cellularity.

FIG. 3. High power of metaphysis shown in Figure 1. Note cellularity and presence of bone on framework of calcified cartilage matrix.

FIG. 4. High power of metaphysis shown in Figure 2 to compare with Figure 3. Note thin bone spicules of cartilage matrix and lack of osteoblasts. Those which are present are spindle-shaped.

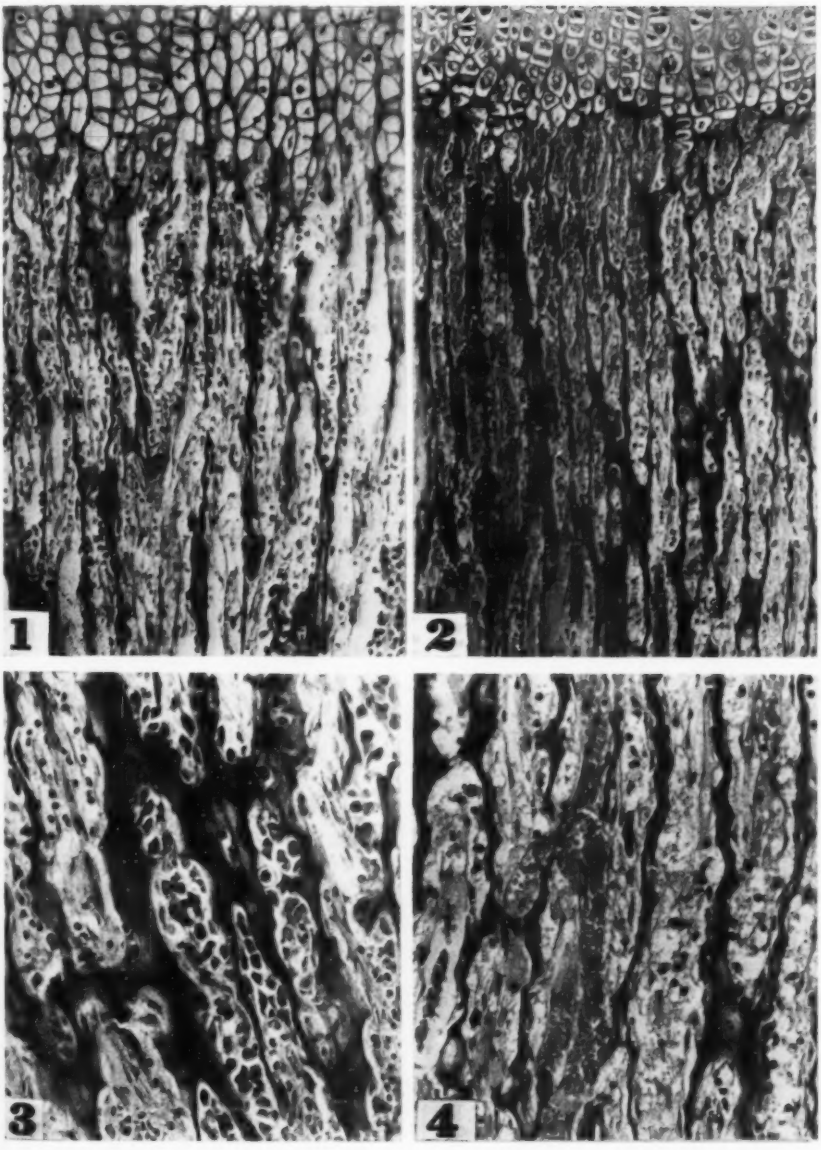
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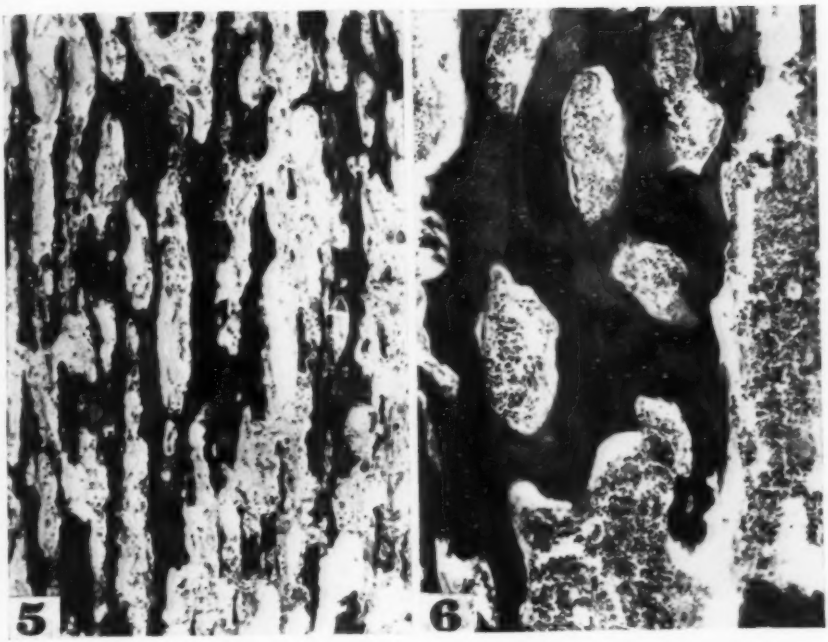
PLATE II

FIG. 5. High power of dense accumulation of calcified cartilage matrix in metaphysis of femur of animal No. 47. There is virtually no bone. Nor are cells prominent.

FIG. 6. High power of area of metaphysis showing disorderly arrangement of calcified cartilage matrix surrounded by bone of animal No. 47.

FIGS. 7 and 8. Cross sections of cortices (mid diaphysis) from control and copper deficient animals, respectively. The only difference is in their width. The size and number of the osteones are similar.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DEHYDROEPIANDROSTERONE, ANDROSTERONE AND 17-HYDROXYCORTICOSTEROID LEVELS IN MATERNAL AND CORD PLASMA IN CASES OF VAGINAL DELIVERY[1,2]

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Gemzell (1) and Bayliss *et al.* (2), using the method of Nelson and Samuels (3), and Robinson *et al.* (4), using the technique of Silber and Porter (5), found an increase in plasma 17-hydroxycorticosteroids during normal pregnancy. Klein *et al.* (6) have reported levels of free corticosteroids in cord blood within the normal adult range, followed by a definite downward trend during the first few days of life. On the other hand, Gardner and Walton (7) observed that the levels of neutral 17-ketosteroids in the cord blood were higher than those in the maternal blood at the time of delivery. Using a specific method for the measurement of dehydroepiandrosterone and androsterone (8), we have studied the levels of these steroids in maternal and cord plasma and correlated them with those of free 17-hydroxycorticosteroids in the same samples. The results indicate that the fetal cortex has functions which differ from those of the glands of the normal adult or the pregnant mother.

METHODS

The subjects in the present study were 30 normal pregnant females from whom venous blood specimens were collected in heparinized tubes at various times close to delivery. Cord blood was also collected just after section of the cord. The plasma was immediately separated from the red cells and stored in a deep freeze until analyzed for steroid content. The length of labor was variable from 1½ to 10½ hours and in case no. 27, 25 hours. Except for patients nos. 14, 15, 16, who received a spinal pentocaine anesthesia prior to an elective Cesarean section, a non-complicated vaginal delivery was performed. The parturients received nitrous oxide and oxygen and had a pudendal block (1% procaine) followed by an episiotomy. Only patients nos. 6, 12, 19, 22, 25, 26 did not require an episiotomy. All the babies were at term, had a body weight in the normal limits, were in good condition at birth and did not need resuscitation.

Free 17-hydroxycorticosteroids (17-OH-CS) were determined by the method of Nelson and Samuels (3). Dehydroepiandrosterone (DHA) and androsterone levels in the plasma were determined by the technique described by Migeon and Plager (8).

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[1] These studies were reported in part at the 13th M. & R. Pediatric Research Conference on Adrenal Function in Newborns and Infants, Syracuse, N. Y. October, 1954.

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TABLE I

Levels of DHA and Androsterone in Maternal and Cord Plasma, When the Mother Sample was Collected After Delivery

Case No.	Time of Maternal Blood Collection, Postpartum	Time of Delivery	Subjects	Plasma 17-KS (μg/100 ml.)		DHA/Andro. Ratio
				DHA	Andro.	
25	7′	6:01 P.M.	Mother V. H. (23 yrs.)	12	—	—
			Baby girl	26.5	8.5	3.1
5	9′	12:59 P.M.	Mother D. Z. (22 yrs.)	33	20	1.6
			Baby boy	42	16	2.6
9	10′	2:10 A.M.	Mother V. S. (26 yrs.)	17	8	2.1
			Baby girl	69	5	14
1	10′	1:46 P.M.	Mother K. McC. (36 yrs.)	9	12	0.7
			Baby girl	36	9	4.
2	15′	10:20 A.M.	Mother S. T. (30 yrs.)	4	11	0.4
			Baby boy	37	18	2.1
27	20′	12:55 A.M.	Mother A. G. (28 yrs.)	6	—	—
			Baby boy	38.0	—	—
26	39′	9:21 P.M.	Mother L. D. (31 yrs.)	5	8	0.6
			Baby girl	15	9	1.7
6	10 hrs.	9:39 A.M.	Mother M. B. (17 yrs.)	10	17	0.6
			Baby boy	22	13	1.7
8	2 days	5:10 A.M.	Mother M. L. (33 yrs.)	17	15	1.1
			Baby girl	30	8	3.8

RESULTS

In 7 cases of child birth, maternal samples were collected from 7 to 39 minutes, and in one instance 10 hours, following delivery (Table I). In 6 other cases, maternal blood samples were withdrawn 8 to 16 minutes prior to delivery (Table II). The time of collection of maternal blood in relation to delivery did not seem to produce any change in the general trend: in all cases, maternal DHA levels were lower than those of the corresponding cord plasma, while androsterone levels were not significantly different, and therefore the ratio DHA/androsterone was higher in cord plasma. In 11 out of 13 cases the DHA levels of the mother were less than 23 μg. per 100 ml. of plasma, one standard deviation less than the mean value for a group of normal non-pregnant females (9). Even 14 and 17 days prior to delivery the DHA concentrations in maternal plasma were less than normal, showing that this phenomenon is not related to the onset of labor (Table II). The sex of the

TABLE II

Levels of DHA and Androsterone in Maternal and Cord Plasma, When the Mother Sample was Collected Before Delivery

Case No.	Time of Maternal Blood Collection, Antepartum	Time of Delivery	Subjects	Plasma 17-KS (μg./100 ml.)		DHA/Andro. Ratio
				DHA	Andro.	
23	8′	10:05 A.M.	Mother L. B. (20 yrs.)	23	18	1.2
			Baby girl	28	14	2.0
24	8′	7:10 A.M.	Mother P. R. (25 yrs.)	16	9	1.7
			Baby boy	46	11	4.1
12	10′	12:35 A.M.	Mother C. R. (24 yrs.)	17	—	—
			Baby boy	27	9	3
11	15′	3:50 A.M.	Mother C. F. (21 yrs.)	17	—	—
			Baby boy	25	—	—
3	15′	2:16 A.M.	Mother I. McK. (27 yrs.)	8	23	0.3
			Baby boy	34	18	1.9
4	16′	7:02 A.M.	Mother L. B. (29 yrs.)	33	20	1.6
			Baby girl	59	25	2.4
8	14 days	5:10 A.M.	Mother M. L. (33 yrs.)	20	—	—
			Baby girl	30	8	3.7
10	17 days	3:40 P.M.	Mother T. H. (30 yrs.)	11	13	0.8
			Baby girl	45	10	4.5

infant did not seem to have any influence on the levels of DHA and androsterone in cord plasma, although the DHA values in females were somewhat more scattered (Fig. 1).

The levels of 17-OH-CS in maternal and cord plasma were compared in 24 cases as shown in Table III. In 21 instances 17-OH-CS levels were much higher in maternal plasma; in cases no. 6 and 8, maternal plasma collected 10 hours and 2 days after delivery respectively, showed values approaching normal; in case No. 21, where the values for maternal and cord blood were inverse to the others, it seems probable that a technical error was responsible for this result. In all other instances, maternal values were between 2 and 4.5 times higher than cord values (Fig. 2). In cases No. 8, 10, 17, 28, 29 and 30 a determination was made at 39 weeks of pregnancy and the levels were respectively 17, 33, 49, 32, 26 and 32 μg. per 100 ml. of plasma. These blood samples were collected between 9:30 A.M. and 11:00 A.M., and were significantly higher than normal control levels at this time of the day. These values, however, were lower than those in the maternal plasma at the time of delivery.

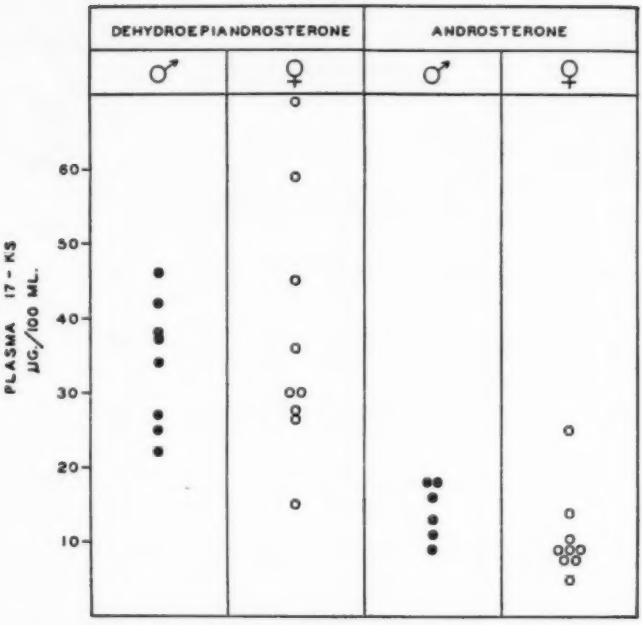

FIG. 1. Comparison of plasma levels of DHA and androsterone in female and male infants. The sex of the infants did not seem to significantly influence these levels.

DISCUSSION

It is commonly considered that only active products which are found in increased amount in the efferent vein of an endocrine gland can be considered as hormones. In pregnancy, mother and fetus, who might produce various steroid hormones, are separated by the placenta, an organ probably able itself to secrete such substances and at the same time play a role of selective "barrier" between mother and fetus. This explains the numerous difficulties encountered in the elucidation of the origin of steroid hormones during pregnancy.

The 17-ketosteroids in cord blood are not free since all samples of plasma were extracted with chloroform before adjustment to acid pH. It is impossible with the information at hand to determine with certainty whether the fetal cortex is producing excessive quantities of the precursor of the conjugated DHA, or whether the high concentrations are due to accumulation in the fetal circulation. Certain points, however, favor the first hypothesis. One of them is the observation of Eagle (10) that the test for DHA of Dirscherl and Zilliken (11) was often positive in extracts of the urine of premature infants. It has also been found that daily output of 17-ketosteroids is relatively high in the first week of life but is followed by a downward trend (12). A similar pattern was observed for the levels of plasma 17-KS (13).

TABLE III

Levels of 17-OH-CS in Maternal and Cord Plasma

Case No.	Time of Maternal Blood Collection	Time of Delivery	17-OH-CS (μg. per 100 ml.)	
			Maternal	Cord
23	8′ antepartum	10:05 A.M.	75	20
24	8′ antepartum	7:10 A.M.	70	30
30	10′ antepartum	11:50 P.M.	54	22
12	10′ antepartum	12:35 A.M.	62	23
11	15′ antepartum	3:50 A.M.	51	17
17	60′ antepartum	10:10 P.M.	81	19
7	90′ antepartum	3:35 P.M.	30	14
18	5′ postpartum	10:37 P.M.	35	8
19	5′ postpartum	3:50 P.M.	42	18
20	6′ postpartum	5:32 A.M.	75	28
21	6′ postpartum	9:24 A.M.	7 (?)	43 (?)
25	7′ postpartum	6:01 P.M.	60	18
22	8′ postpartum	2:00 P.M.	58	2
28	9′ postpartum	10:22 P.M.	76	22
5	9′ postpartum	12:59 P.M.	23	6
9	10′ postpartum	2:10 A.M.	62	26
27	20′ postpartum	12:55 A.M.	75	34
26	39′ postpartum	9:21 P.M.	83	10
29	53′ postpartum	5:52 P.M.	69	20
6	10 hrs. postpartum	3:39 A.M.	18	13
8	2 days postpartum	—	13	23
14	1 day before Cesarean	8:15 A.M.	46	3
16	60′ before Cesarean	8:00 A.M.	39	0
15	3 days after Cesarean	8:03 A.M.	43	15

The 17-KS variation in urine and plasma seems to parallel the involution of the inner layer of the adrenal cortex (14). Recently the presence of Δ_4-androstene-3,17-dione in fetal adrenals was demonstrated (15). These findings would tend to show that the fetal adrenal can probably produce androgens and that the urinary 17-KS excretion in the newborn is related to adrenal secretion. The fact that newborn females have levels of plasma and urinary 17-KS similar to those of the males would tend to eliminate the testis as a major site of androgen formation at this time.

If fetal adrenals produce androgens *in utero*, one is brought to wonder how these steroids are eliminated from the fetus. In adults, steroids are known to have a rapid turnover and hormonal levels in the body are maintained by continuous production. We have not been able to find any 17-KS or 17-OH-CS in amniotic fluid. It would, therefore, be logical to think that the mother excretes the metabolites of androgens produced by the fetus; the increased excretion of 17-KS by Addisonian mothers might prove this point although one must be aware that 20-ketones can interfere with the Zimmer-

mann reaction as pointed out by Venning (16). Dobriner and Lieberman (17) have observed if anything a slight decrease in the urinary excretion of androsterone and etiocholanolone toward the end of pregnancy. If one assumes that the fetal adrenals can produce androgens, and that their metabolites are excreted by the mother, one would have to postulate that the maternal production is decreased.

As far as the 17-hydroxycorticosteroids are concerned it appears that pregnancy can raise the plasma levels of the mother (1, 2, 4) and labor can raise these levels further as the very high values reported in this paper would tend to demonstrate. Cord plasma levels would appear to be conditioned largely

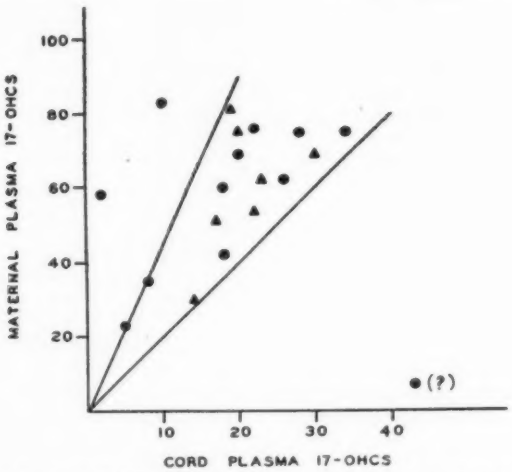

FIG. 2. Comparison of 17-OH-CS levels in maternal plasma and cord plasma. In most instances maternal levels were between 2 and 4.5 times higher than the respective cord levels. (▲: antepartum ●: postpartum. Only cases where maternal sample was collected 90′ or less before or after delivery are plotted on the figure.)

by maternal concentrations. It is interesting to note that in every instance, with the exception of one dubious value, 17-OH-CS levels in maternal plasma were higher than those in cord plasma.

SUMMARY

The levels of DHA, androsterone, and 17-OH-CS in maternal and cord plasma were determined in cases of vaginal delivery.

As in the normal adult, the 17-ketosteroids were conjugated in a form split by weak acid (pH 0.8). DHA levels in cord plasma were similar to those found in normal adult individuals, but higher than those of the respective mothers. Androsterone levels in maternal and cord plasma were similar to those found in normal adult individuals.

In most instances 17-OH-CS levels in cord plasma were somewhat higher than the normal adult average for the corresponding time of the day, but were lower than those of the respective mother.

The meaning of these findings in terms of the functions of the fetal and maternal adrenal cortices is discussed.

ACKNOWLEDGEMENT

The authors wish to express their grateful appreciation to Dr. Leo T. Samuels for his great interest in this work.

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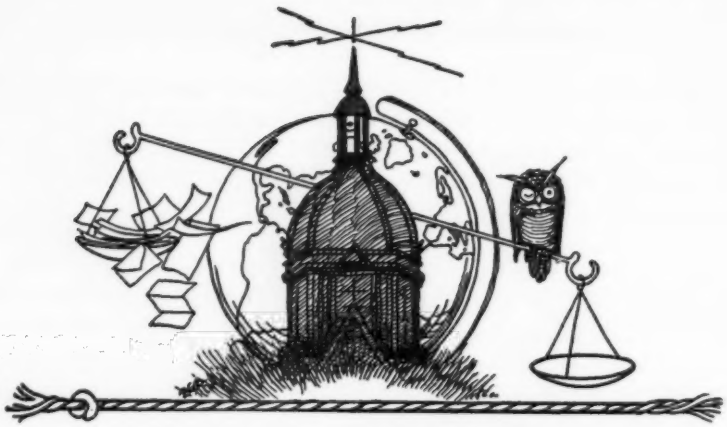

BOOK REVIEWS

Clinical Measurements of Uterine Forces in Pregnancy and Labor. By S. R. M. Reynolds, Jerome S. Harris and Irwin H. Kaiser. 328 pp., $9.50. Charles C. Thomas, Springfield, Illinois.

Three basic components are involved in the mechanisms of labor and delivery of the infant, namely, the measurement and capacity of the pelvis, the size of the infant's head, and the forces of labor. The clinical obstetrician at the present time can only obtain accurate measurement of the pelvis by x-ray pelvimetry. Calculation of the infant's head measurements before delivery are only reasonable approximations by the clinician. Detection of the forces of uterine contractions has been determined by direct palpation. Various devices have been devised over the past one hundred years to measure uterine forces, but they have not been of practical value to the obstetrician. During the past decade improvements in electrophysiological equipment have renewed interest in the measurement of the uterine forces in pregnancy and labor.

The three authors of this volume have played a major role in the development of these new instruments. After World War II numerous technical advances led to the development of a satisfactory tokodynamometer by the authors and various colleagues. Based on their experiences with this instrument and the experiences of others, a need for a collation and standardization of terms, nomenclature and procedures in this new science was needed.

The book is divided into four parts with a total of fourteen chapters. Part one reviews the interesting early history of tokodynamometry, as well as the physiological principles involved. In part two basic methods such as the intraovular, transcervical and extraovular types are described. The details of the preparation and management of the patient, and numerous recordings made with these instruments are profusely illustrated with charts and photographs. Part three of the volume is concerned with uterine contractions during pregnancy and labor. Excellent discussions of the numerous factors involved such as frequency, duration, effect of parity and many others during the various stages of labor are reviewed in these chapters. Part four will be of special interest to the practicing obstetrician, since the effects of the psyche and the actions of various drugs on uterine contractions are thoroughly discussed. Many debatable clinical points such as the effects of analgesia and anaesthesia on uterine contraction are raised. The authors of this volume present their views on this subject. More specific effects such as those of adrenalin and levo-epinephrine are well illustrated.

Perhaps the most significant clinical discussion is in the concluding chapters which deal with the use and abuse of pitocin. The action, dosage, routes of administration, indications, and contraindications of this valuable aid to obstetrics is thoroughly presented.

In general, this volume as was the purpose of the authors in writing it, will prove to be of use to teachers and investigators as well as the practicing obstetrician in fostering a better understanding of the uterine forces in pregnancy and labor.

GEORGE W. ANDERSON

Laboratory Identification of Pathogenic Fungi Simplified. By ELIZABETH L. HAZEN AND FRANK CURTIS REED. 108 pp., $5.50. Charles C Thomas, Springfield, Illinois.

This monograph is a compact and much simplified manual covering the laboratory findings and procedures relevant to the pathogenic fungi most commonly found in North America. The authors have condensed the material remarkably well. For instance, the pertinent clinical and laboratory data are summarized for each fungus on a single page. Its characteristic macroscopic and microscopic features are illustrated in well-chosen photographs on another page. At the end there is a list of references, and formulas for some of the media. It should serve as an excellent guide for teaching purposes, as well as for the isolation and identification of fungi.

A. M. FISHER

Talking with Patients. By BRIAN BIRD. 154 pp., $3.00. J. B. Lippincott Co., Philadelphia, Pa.

This gem of a book is a small collection of subjects totaling one hundred and forty-four pages, discussing the various human problems involved in doctor-patient relationships. It is not a technical discussion. In fact it is a very simply worded, common sense book. It is of a special value to any physician in clinical work, as well as, to medical students, nurses and others who deal with patients.

The author begins with discussing defenses as part of illness. He discusses illness for pathology as a defense. He goes on to discuss history taking technique and various types of patients with various types of emotional attitudes toward the meeting with the doctor. There is an interesting chapter on giving advice which is quite timely and certainly is a valuable bit of observation of human nature. Other chapters include those on suicide, grief, the mentally ill patient, the mentally retarded patient, the anxious patient, the anxious doctor, a most important discussion of fees, various stages of childhood development in doctor-patient relationships, reaction of a child to anesthetic, reaction to injury, separation from parents on the part of a child, a child's special language, sex problems and finally a most important chapter on dealing with the parents of the patient.

This book covers in economical language a wide range of problems involved in talking with patients. It stresses throughout, that the doctor, himself, is a human being, with human reactions and feelings; and it is important that he have some awareness of how his feelings enter into the treatment situation whether he be a surgeon, a psychiatrist or a person doing full time work in a teaching hospital.

The information given and the points of view presented in this book are far from dogmatic and yet in its general tolerant approach it gives something to the reader which is well worth taking with him. It represents an attempt and a quite successful one to deal with the human element of the doctor-patient relationship. It deals specifically with the emotional attitudes both those manifest and those hidden, which both the doctor and the patient commonly show.

WILLIAM T. DIXON

The Practice of Dynamic Psychiatry. By Jules H. Masserman. 790 pp., $12.00. W. B. Saunders Co., Philadelphia, Pa.

In an earlier book (Principles of Dynamic Psychiatry, 1946) the author of the present volume formulated a comprehensive system of physiologic and psychologic concepts of behavior for which he coined the term *biodynamics*. *The Practice of Dynamic Psychiatry* is an extension, and to some extent an elaboration, of the ideas set forth in the earlier work, applying those constructs "to clinical psychiatry" and to the theory and practice of medicine and its specialties."

The book is divided into five parts and an appendix. Part I, *The Clinical Investigation of Human Behavior*, deals lucidly and excellently with the psychiatric interview as well as various techniques, including formal psychologic testing methods, currently held useful in appraising personality functioning.

Part II, *Dynamic Evaluation of Clinical Syndromes*, is concerned with a description of various types of disordered behavior and makes use of the 1952 revision of Standard Nomenclature of Nervous and Mental Diseases. Although recognizing the artificiality of labeling a patient's symptoms as always denoting a nosologically distinct condition, whereas in actual practice one sees countless varieties of the so called normal and idiosyncratic, the author sometimes veers too sharply away from what he disparages as "preconceived empiricism." For this reason many students will prefer, as being more helpful in their early training, the more didactic descriptions of psychiatric disorder to be found in other texts.

Part III, *Case Studies and Communications*, undertakes to illustrate "ways of formulating psychiatric etiology, diagnosis and prognosis in a manner best suited to the respective needs of medical consultants, jurists, insurers and others with whom the physician must deal in his daily practice." The thoughtfully written case reports which the author has included in this part of the book should prove useful models.

Part IV, *Clinical Dynamics of Affect and Self*, sets forth the authors position in psychiatric theory. In addition, this section contains an interesting review of the history of psychiatric theory ranging from primitive animistic beliefs through theories and hypotheses currently in vogue.

Part V, *The Practice of Dynamic Psychotherapy*, continues this historical survey as it applies to treatment. Bulking nearly half the total pages in the book, this section, in the author's opinion, is its most important part. In Part V the author discusses current psychodynamic concepts, particularly those of psychoanalysis, with unusually candid perspicacity. His delightful essay, "The Psychosomatic Profile of an Ingrown Toenail" (page 474) is a healthy antidote for the flights of fantasy too frequently encountered in present-day psychiatric writing. It would be satisfying to be able to report that the author had avoided all the pitfalls of like nature himself. To this reviewer some of the author's "biodynamic" concepts appear so little changed from the more familiar psychoanalytic ones that they might just as well have been left as they were, or, where more cogent data contradicts, dismissed. There is a bit too much of trying to make the divergent schools of psychiatric theory fit neatly together, whereas it is not unlikely that the advance of knowledge will one day require many current day concepts to be discarded as nonsense. Even so, Part V, like the rest of the book, is so well written and contains such instructive perspectives that it more than lives up to the author's opinion of its preeminence in the book as a whole.

There is a useful appendix which contains a potpourri of information of varying usefulness. The undergraduate psychiatric curriculum as outlined on page 688 is thought-provoking as a serious minded effort to bring psychiatry, head erect, out of the academic corner in which it cringes in many medical schools today. An impressive 48 page, up-to-date bibliography concludes the book.

John L. Hampson

Annual Review of Medicine, Vol. VI. Edited by DAVID A. RYTAND. 459 pp., $7.00. Annual Reviews, Inc., Stanford, California.

The Annual Review of Medicine has become a most helpful contribution to medical literature. Its chief use lies perhaps in its value as a reference book for quick synopsis and for access to the recent literature on a given subject. Also in this volume there are 35 pages of an annotated list of reviews on various medical subjects. This list alone is most worthwhile and should be known to all.

The Annual Review of Medicine would be rather difficult to assimilate if one read directly through the book. But as a reference book it is most valuable in analysis of disease problems or in teaching or in reviewing a specific field of medicine.

Special chapters in this volume are: "Contrast Methods in Diagnostic Roentgenology", "Antihypertensive Drugs", "Toxicology" (on Poisons), and "Recent Advances in Ophthalmology".

Most physicians will want to read the chapter on viruses by Scott because it contains much important new material of interest to all, and not just to virologists. Likewise the chapter on the liver by Bearn and Kunkel is helpful in giving a well-balanced modern view of abnormal liver processes. A discussion is given of the unusual incidence of cirrhosis in young females, observed now for a number of years. The newer findings in Wilson's disease are brought together.

Maloney and Blalock have a timely well-written chapter on cardiovascular surgery, recommended for all because of the enhanced therapeutic possibilities in this field. The chapter on allergy and immunology by Cooke, Smith, and Skaggs is most stimulating and indicates some of the noteworthy progress being made in this broad field.

In the chapter on cancer the role of the carcinogens is thoroughly discussed by Haddow. An excellent review of the use of antihypertensive drugs is made by Smirk.

The scope of medicine today makes this a most useful yearly addition to review-type of literature and a volume needed for frequent ready reference.

F. W. BARNES, JR.

Letalfaktoren in ihrer Bedeutung für Erbpathologie und Genphysiologie der Entwichlung. By ERNST HADORN. 338 pp. $9.30. Georg Thieme Verlag, Stuttgart, Germany. Also Intercontinental Medical Book Corp., New York, N. Y.

This monograph should serve a dual function. Since it enumerates the causes and records the developmental deviations of many genetically-induced pathological conditions found in a variety of animals throughout the animal kingdom, it sheds considerable light upon, and thereby clarifies, many fundamental problems of normal genic action and of embryogenesis. The monograph, further, by virtue of its inclusion of many examples of lethal and sub-vital conditions found in man, may stimulate clinical genetics along the lines of investigation and analysis presented here.

Dr. Hadorn, Professor of Zoology and Comparative Anatomy at the University of Zurich, is in a good position to have undertaken such a book since he has played a leading role in the field of biochemical and physiological genetics during the past twenty years and has devoted himself particularly to a study of lethal genetic effects. Although written primarily for geneticists, there is much to recommend the monograph to all those interested in the problem of abnormal physiological and organic development.

Lethal factors are discussed in a general way as deleterious Mendelian or chromosomal defects with various degrees of expression in the phenotype. They occur either naturally or as experimentally-induced mutations within every group of animals thus far subjected to genetic analysis. The penetrance of a lethal factor in the genotype varies from a small degree of incidence to one hundred per cent expression. Its manifestation, however, can be altered

by various external conditions such as severe changes in temperature, nutrition and population density, as well as by internal factors pertaining to the general genotypic milieu.

The effective period of action of a lethal factor may act at any time during the embryonic, post-embryonic or juvenile stage of development, and while most lethal genes are quite specific in their period of action, others are polyphasic and act at several distinct times during embryogenesis. Moreover, the effects of any given lethal factor are generally limited to a particular cell type such as blood cells, rod cells or spinal cord, but occasionally the destructive effects are multiple. The significance of dominance, recessiveness, phenocopy, pleiotropy, polygenes and chromosomal rearrangement is considered in detail in respect to the appearance of lethal characters in man and in infrahuman species.

In the sections which deal principally with human heredity Professor Hadorn discusses the calculated mutation rates of a dozen lethal genes and he indicates that, while the number of afflicted young born may be reduced by restricted breeding, few if any of these factors will ever be eradicated owing both to new mutations and to genetic masking of undetectable genes. He has traced the probable mechanisms of transmission for a number of factors associated with human pathological conditions, including sickle cell anaemia, thalassemia (Cooley's anaemia), telangiectoma, chondrodystrophy, dominant brachydactyly and xeroderma pigmentosum. He notes the useful experimental fact that two human blood anomalies have identical defects in laboratory animals, haemophilia in the dog and the dominant semi-lethal character which hinders segmentation of neutrophilic nuclei in the rabbit.

The author seems most at home in his presentation of recent work, much of it his own, concerning the biochemical and physiological changes associated with conditions attributable to lethal factors. Indeed, the profound alterations in such metabolic components as free amino acids, enzyme systems, hormones, oxygen consumption and fluid balance in genetically-induced anomalous individuals as compared with "genetically normal" members of the same species indicate a new frontier in the study of the early development of pathological diathesis. While most of these investigations have been conducted on lower animals their application to man should lead to greater understanding, to earlier detection, and probably to more frequent correction of human hereditary syndromes.

DAVID W. BISHOP

Glandular Physiology and Therapy. A Symposium, 5th edition. Prepared under the auspices of the Council on Pharmacy and Chemistry of the American Medical Association. 611 pp., $10.00. J. B. Lippincott Co., Philadelphia, Pa.

The various sections of this book were written by thirty-one contributors. In this 5th edition, appearing twelve years after the 4th, there are excellent chapters covering most aspects of endocrinology. Emphasis is placed, as the title indicates, on physiology of the glands of internal secretion and in therapy of endocrine disease. Due to excellent editing the book has good continuity, a minimum of reduplication, and accurately compiled references. It makes pleasant reading while also serving as a reference source.

Errors and inconsistencies appear as might be anticipated in so large an undertaking. For example, the statement on page 573 that thyroxine is a protein with molecular weight of about 675,000 is incorrect, for thyroxine is an amino acid with molecular weight of 777. The author is undoubtedly referring to thyroglobulin. On the whole, however, such errors are rare.

In the preface it is suggested that this may be the last edition to be published, because the needs of the field are now served by several textbooks of endocrinology. This would be regrettable, for this book is in itself an excellent text.

S. P. A., JR.

Regional Enteritis. Diagnostic and Therapeutic Considerations. By FREDERICK F. BOYCE. 84 pp., $2.35. J. B. Lippincott Co., Philadelphia, Pa.

When one reviews a play for the lay press, he's inclined to recommend it if he has truly enjoyed the performance, that is, if he has been emotionally in accord with the action of the drama and if the actors have done their parts with a technique which made their characters alive. And your reviewer is eager to acclaim Dr. Boyce's little monograph which has seemed to him to have been soundly based on the author's personal experience with regional enteritis as well as on the literature of the disease. It tells clearly what is known of this increasingly observed intestinal difficulty and it recounts it in a style that is not tiring to read. Indeed, all its sixty-nine small pages might be read at a single evening session; and that's something which can't be said of many texts!

The author gives considerable space to the investigations into the psychosomatic studies of the cause of regional enteritis and then wisely inserts one of his own comments on the psychiatric approach to the etiology. He says, "This is entirely in line with the modern tendency to emphasise psychosomatic origins before organic causes have been excluded. This course provides a quick way out of diagnostic difficulties but makes a good deal of later trouble for other physicians and their patients." And the work contains frequent similar leveling comments.

Your reviewer was surprised to learn that Shields Warren and S. C. Sommers have not regarded gross ulceration as uniformly characteristic of regional enteritis; he has considered ulceration to be present in all cases of the disease who have symptoms. And the reviewer could not agree with the statement concerning the diagnosis to the effect that a positive roentgenogram is conclusive. Two pages later this is properly contradicted by "Sometimes the diagnosis is in error in spite of the roentgenologic findings."

But the monograph is nevertheless a good one and it is recommended without reservations to those who wish to refresh their clinical knowledge of regional enteritis.

JOHN TILDEN HOWARD

BOOKS RECEIVED FOR REVIEW

Cancer Cells. By E. V. COWDRY. 677 pp., $16.00. W. B. Saunders Co., Philadelphia, Pa.

Human Pathology, 8th edition. By HOWARD T. KARSNER. 960 pp., $15.00. J. B. Lippincott Co., Philadelphia, Pa.

Office Procedures. By PAUL WILLIAMSON. 412 pp., $12.50. W. B. Saunders Co., Philadelphia, Pa.

Orthopädische Krankengymnastik. Leximon und Kompendium. By MARTHA SCHARLL. 174 pp., kartoniert DM 14.80 ($3.50). Georg Thieme Verlag, Stuttgart, Germany. Also Intercontinental Medical Book Corp., New York N. Y.

Premature Infants. A Manual for Physicians, 2nd edition. By ETHEL C. DUNHAM. 459 pp., $8.00. Paul B. Hoeber, Inc., New York, N. Y.

Sexual Precocity. (American Lecture Series). By HUGH JOLLY. 276 pp., $6.75. Charles C Thomas, Springfield, Illinois.

Surgery of the Ambulatory Patient. By L. KRAEER FERGUSON. 866 pp., $12.00. J. B. Lippincott Co., Philadelphia, Pa.

Textbook of Endocrinology, 2nd edition. Edited by ROBERT H. WILLIAMS. 776 pp., $13.00. W. B. Saunders Co., Philadelphia, Pa.

Transactions of the 14th Conference on the Chemotherapy of Tuberculosis. Prepared and edited by the Veterans Administration Area Medical Office, Atlanta, Ga. and the Department of Medicine and Surgery, Central Office, Washington, D. C.

Wiederbelebung. By H. KILLIAN AND A. DÖNHARDT. 320 pp., Ganzleinen DM 24. ($5.70). Georg Thieme Verlag, Stuttgart, Germany. Also Intercontinental Book Corp., New York, N. Y.

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BENJAMIN FRANKLIN

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OTHO T. BEALL, JR. AND RICHARD H. SHRYOCK

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Another objective of the authors has been to provide a detailed case study of medical thought in America at the beginning of the Eighteenth Century, a period hitherto largely overlooked as making any contribution to the field. Because this disregard has been the result of scarcity of materials, Mather is a particularly valuable source; he provides an almost unique mass of evidence concerning early American medicine, with all its implications for the social and cultural history of the times.

In the appendix will be found selected sections from Mather's chief medical work, "The Angel of Bethesda," here printed for the first time.

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Deca-Vi-Sol—the new, more comprehensive formula including vitamins B_{12} and B_6—permits even greater flexibility in specifying vitamins for infants and children. Like Poly-Vi-Sol and Tri-Vi-Sol . . .

Deca-Vi-Sol is . . . **highly stable** . . . refrigeration not required . . . potency assured . . . **readily accepted** . . . exceptionally pleasant flavor . . . no unpleasant aftertaste . . . **full dosage assured** . . . can be dropped directly into the baby's mouth.

For older children, specify **Mulcin,** the good-tasting, orange-flavored vitamin liquid for teaspoon dosage.

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MEAD JOHNSON & COMPANY • EVANSVILLE, INDIANA, U. S. A.

Vitamin A (units)